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Endogenous Formation of Carcinogenic N-Nitrosamines from Dietary Nitrite: Mechanisms, Modulators, and Cancer Risk

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ABSTRACT

Nitrite dietary intake is utilized widely in the curing and processing of meats for their antimicrobial action, *Clostridium botulinum* inhibition, as well as for their role in imparting meat color and flavor. Yet, nitrite can become involved in endogenous N-nitrosation, resulting in the formation of N-nitrosamines and other N-nitrosocompounds (NOCs), including N-nitrosodimethylamine (NDMA), following consumption. The present review focuses on the chemistry, gastrointestine physiology, microbial action, toxicity, epidemiology, and regulation of this phenomenon. Nitrite, in an acidic environment of the stomach, is transformed into nitrous acid and nitrosating intermediates, such as nitrosonium derivatives and dinitrogen trioxide, which react with secondary amines and amides. In cases of hypochlorhydric or achlorhydria, bacterial involvement can provide an alternative mechanism. Additionally, the review also draws a distinction between food sources where, as observed, nitrate-containing vegetables make an important contribution to the body's nitrate-nitrite burden, although their high level of vitamin C and polyphenols would inhibit nitrosation. Some other sources include heme iron, thiocyanates, carbonyl compounds, dietary amines, and selected microbial processes that facilitate the production of NOCs while ascorbate, tocopherol, polyphenols, and thiols prevent this reaction from taking place. Once metabolically activated, NDMA may be involved in the creation of DNA reactive electrophiles such as methyl diazonium that lead to mutagenic DNA adducts such as O6-methylguanine. The epidemiological observations differ depending on the type of cancer and source of exposure, with more consistent results being obtained for gastric cancer as compared to colorectal, oesophageal or pancreatic cancer. The review also mentions limitations of concentration-based assessments and suggests several methods such as source-resolved exposure assessment, biomarkers, physiologically based pharmacokinetic models and nitrosation potential index. Special emphasis is placed on the difference between chemical plausibility, biomarker data, epidemiological observations and causal inference.

Keywords: Dietary Nitrosamine, Endogenous nitrosamine, Nitrate, Nitrile.

1. INTRODUCTION

1.1. Preservatives

Preservatives are chemicals added to food to prolong its shelf life while preserving its safety, quality, and nutritional content by preventing microbiological spoiling and delaying chemical deterioration (El Alami El Hassani et al., 2025). For generations, meat has been preserved and cured using nitrite salts, which are prized for their capacity to prevent the growth of *Clostridium botulinum*, maintain the pink type of cured goods, and add to their unique flavour. This preservative function is still very essential because nitrite curing is one of the best and most researched ways to prevent botulism in processed beef, and removing it without a suitable replacement comes at a cost to food safety. However, the same chemistry that makes nitrite an efficient antibiotic also makes it a precursor to a class of substances known to provide a unique and mostly distinct risk: N-nitroso compounds (NOCs), some of which are known carcinogens, including N-nitrosodimethylamine (NDMA) (Zhong et al., 2024).

1.2. Dietary nitrite preservatives

No single alternative preservation technique can equal nitrite's combined effectiveness against oxidative rancidity, pathogenic spoilage organisms, and botulinum outgrowth at a cost that is economically feasible, making this antimicrobial function extremely difficult to replace. Dietary nitrite exposure is not only through the direct addition of nitrite as a preservative. Fresh, unprocessed foods such as fresh meat, fish, and vegetables generally contain negligible or trace amounts of N-nitrosamines, but processing and cooking significantly increase the amounts formed. Microorganisms also contribute to nitrosation during meat processing by reducing nitrate to nitrite, degrading proteins to amines, and generating nitrosation-promoting enzymes (Zhong et al., 2024). The second, quantitatively larger source of dietary nitrate is vegetables which are subsequently converted to nitrite endogenously via the enterosalivary pathway described in Section 3.2. This means that a substantial share of the body's internal nitrite pool derives from nitrate-rich vegetables rather than added meat preservatives at all. The heterogeneity of these sources has direct implications for the interpretation and regulation of dietary nitrite exposure. Plant-derived nitrate and nitrite are associated with different health outcomes than those found in animal-based foods, processed meats and drinking water. The divergence is due to the presence of nitrosation inhibitors vitamin C and polyphenols in vegetables, which is largely absent from processed meat matrices (Molina et al., 2024). Therefore, the regulatory response has become more stringent specifically around the meat-preservative pathway. The European Union has established a maximum permitted limit for input nitrite at 80 mg/kg for both non-heated and heat-treated meat products, which will be implemented from October 2025, down from the previous limit of 150 mg/kg established under earlier European Parliament and Council Directive standards (Molina et al., 2024).

1.3. Endogenous carcinogenic nitrosamine

An important distinction that must be drawn when considering the risks associated with nitrite intake is that dietary nitrite in and of itself is not a carcinogen but a precursor to the formation of carcinogenic N-nitrosamines. The main hazard posed by dietary nitrite results from the transformation of nitrite into reactive nitrosating compounds in the gastrointestinal tract, and the subsequent reaction of nitrosating agents with secondary amines formed from proteins consumed in food to produce carcinogenic N-nitrosamines. The main nitrosating agents are nitrate and nitrite, while some nitrite also is produced by bacteria inhabiting the saliva from reducing about 25% of ingested nitrate (Zhong et al., 2024). Endogenous production is the main route of nitrosamine exposure and not merely an additional one to preformed nitrosamine exposure – the integration of over half a century of studies on the toxicokinetics of nitrosamines demonstrates that endogenous N-nitrosodimethylamine

(NDMA) exposure might be two to three orders of magnitude higher than preformed NDMA exposure through the diet (Eisenbrand et al., 2024). This discovery has clear regulatory implications, as the existing methodologies for nitrosamines risk assessment, such as the margin-of-exposure approach utilized by the European Food Safety Authority, take into account only preformed nitrosamines contained in food prior to consumption, thus being unable to reveal a hazard developing after food ingestion (Kotopoulou et al., 2022). Indeed, the classification of ingested nitrate or nitrite, in conditions favouring endogenous nitrosation, as probably carcinogenic to humans (Group 2A) by the International Agency for Research on Cancer is based on precisely the same understanding of the hazard posed by nitrites.

2. CHEMISTRY OF N-NITROSATION

2.1. Formation of the nitrosating species

The first step in N-nitrosation is the transformation of consumed nitrite into reactive nitrosating agents in an acidic environment. Nitrous acid (HNO2) breaks down in the acidic stomach environment to produce reactive nitrosating species, mostly nitrosonium ion (NO^+) and dinitrogen trioxide (N2O3). These species are short-lived but highly electrophilic, and their synthesis is largely pH-dependent: the stomach's acidic pH of about 1-3 promotes the quick protonation of nitrite to nitrous acid, which then breaks down to produce the active nitrosating intermediates. This explains why gastric chemistry is increasingly recognised as a primary location of nitrosamine production rather than only food-processing chemistry.

2.2. Competing reaction pathways

The fact that these nitrosating intermediates do not only react with amines is a factor that was frequently overlooked in previous evaluations. The stomach produces reactive nitrosating species that react with a variety of substrates, such as thiols to form S-nitroso compounds, aromatic compounds to form C-nitroso species, and secondary amines and amides to form NOCs, many of which are known or suspected carcinogens. This competition is important for evaluating risk: The amount of ingested nitrite that is eventually directed toward N-nitrosamine formation depends on the relative abundance of competing nitrosatable substrates in the gastric environment at the time of digestion because S-nitrosation of thiol-containing compounds (such as glutathione and cysteine) consumes a portion of the available nitrosating capacity without producing a carcinogenic product (McNicol et al., n.d.). There are different pathway which leads to the carcinogenic end products as shown in Figure 1.

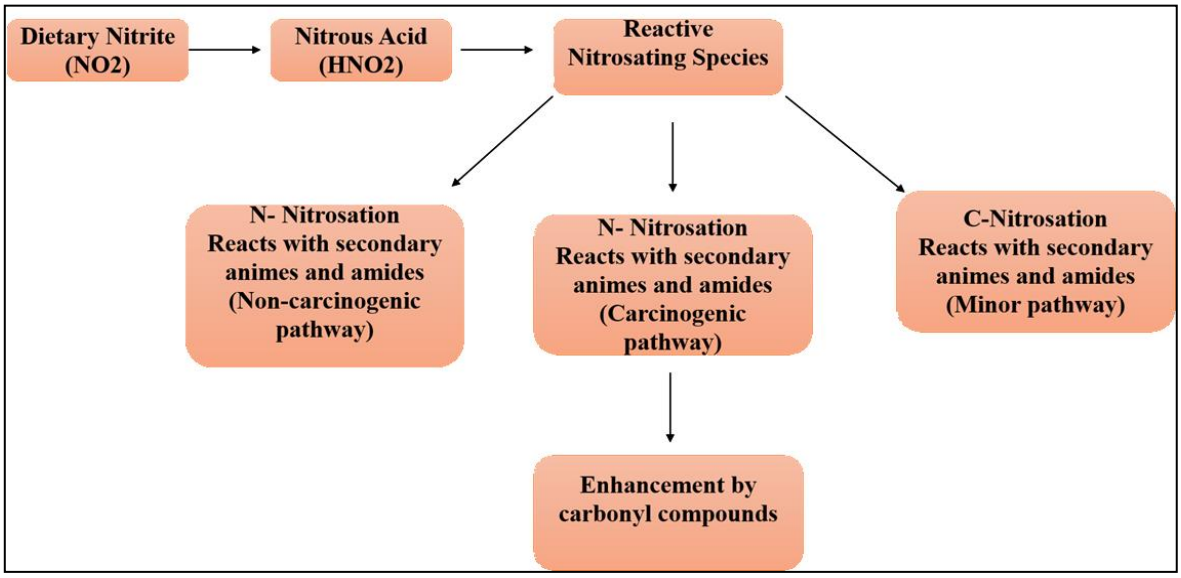


Figure 1: Formation and competing fates of nitrosating species

2.3. The nitrosation reaction with secondary amines

N-nitrosamines are created when nitrosating agents combine with secondary amines in a traditional electrophilic substitution at nitrogen. Secondary amines are the amine class of primary toxicological concern in this pathway because they react with nitrous acid to form N-nitrosamines. This reaction produces a stable, usually oily N-nitroso product, in contrast to the corresponding reactions of primary amines and tertiary amines. Mechanistically, this happens through nitrosonium-mediated nitrosation, which involves acidification of nitrite. This is different from another gas-phase radical pathway where NO2 takes a hydrogen atom from the amine nitrogen to form an aminyl radical–nitrous acid complex, which is then quenched by NO. Alkyl substitution on the amine significantly reduces the activation barrier for this reaction, according to computational work. The hydrogen-abstraction barrier drops from 106 kJ/mol for ammonia to 72 kJ/mol for methylamine and 45 kJ/mol for dimethylamine. This helps explain why dimethylamine, an amine that is frequently found in fish and meat protein breakdown products, is such an effective precursor to NDMA in particular (Zhao et al., 2007).

2.4. Implication for the pharmaceutical and food nitrosamine literature

It is noteworthy that pharmaceutical nitrosamine-impurity research, rather than food science, has driven much of the most recent mechanistic refinement in this field. Density functional theory calculations are used to assess how electronic and steric effects on a variety of secondary amines affect nitrosation rate in the presence of nitrite under acidic conditions. Understanding the critical factors governing N-nitrosation mechanisms and activation energy barriers has become crucial to nitrosamine risk assessment in drug substances. Since the underlying chemistry nitrite plus secondary amine under mildly acidic to neutral conditions is the same whether the matrix is a drug formulation, wastewater, or the human gastric environment, this cross-disciplinary convergence is a true strength for a food-science review to draw from. A review that specifically draws on this pharmaceutical mechanistic literature can offer more rigorous kinetic grounding than most food-science-only reviews currently provide (Kawamura et al., 2025).

3. DIETARY SOURCES AND EXPOSURE ROUTES

3.1. Processed and cured meat as a primary nitrite source

The biggest source of directly added dietary nitrite is still processed and cured pork products, such as bacon, gammon, sausage and their regional equivalents. Due to their role as nitrosamine precursors, this intake is currently tightening under regulatory pressure. The previous European Parliament and Council Directive limited sodium nitrite addition in processed meat to 150 mg/kg, with a residual limit below 100 mg/kg and a higher residual allowance of 175 mg/kg specifically for cured bacon. As of October 2025, the European Union's maximum permitted limit for nitrate ion addition in non-heated meat products is 90 mg/kg, while the limit for input nitrite is 80 mg/kg for both non-heated and heat-treated meat products. The Codex General Standard for Food Additives, which applies to heat-treated processed meat, poultry and game products, is now in line with this tighter EU restriction (Molina et al., 2024).

3.2. Nitrate-rich vegetables and the oral-to-systemic conversion pathway

By far the most common source of dietary nitrate is vegetables. Vegetables account for up to 80–85% of total nitrate intake, with leafy greens and root vegetables having the highest concentrations. Onions, peas, and potatoes have relatively low nitrate levels, whereas beetroot, cress, lettuce, spinach, rucola, and celery may have 1,000–2,000–2,500 mg / kg fresh weight. The enterosalivary pathway is a well-established mechanism for absorption and conversion. Using enzymes that human cells do not have,

bacteria on the posterior tongue transform swallowed nitrate into nitrite. The nitrite is then swallowed and further transformed into NO and related nitrogen species in the acidic gastric environment the same acid-driven chemistry that is responsible for the formation of nitrosating species as discussed in Section 2. Nitrate bioavailability from plants is high (98±12% from cooked spinach, 114±14% from raw lettuce, and 106±15% from cooked beetroot), with an estimated 4–8% of the ingested nitrate dose being converted to nitrite, according to pharmacokinetic study. Since antibacterial mouthwash can completely stop the entero salivary loop, interrupting this oral bacterial phase alters systemic exposure (Velzen et al., 2008).

3.3. Drinking water and minor sources

The total lack of co-occurring nitrosation inhibitors sets drinking water apart from vegetable-derived nitrate, making it a quantitatively smaller but increasingly scrutinised source of nitrate. As one water-quality expert put it, water provides. In the past, regulatory restrictions have been established for an endpoint other than cancer. The US EPA sets a maximum contaminant level of 10 mg/L nitrate-nitrogen (or 44.3 mg/L nitrate) for drinking water nitrate. This limit is largely intended to avoid infant methemoglobinemia ("blue baby syndrome") rather than cancer risk. According to recent cohort data, this threshold might not be particularly protective against cancer (Mendy & Thorne, 2024). A related analysis revealed that detectable nitrate in drinking water was linked to a 73% increased risk of cancer mortality, with risk rising 69% for each 10-fold increase in concentration effects seen even at levels below current EPA limits. A 2024 US cohort study concluded that drinking-water nitrate levels may be an overlooked contributor to cancer mortality. Gastric cancer risk nearly doubled for every 10 mg/L increase in drinking-water nitrate, according to a review of 60 studies. Similarly, a large. The cohort found that drinking-water nitrate concentrations ≥9.25 mg/L were linked to a 52% higher colon cancer rate, which is significantly below the EU's regulatory limit of 50 mg/L (Palmero et al., 2026).

3.4. Plant vs animal source distinction as an epidemiological confound

This has been directly shown in recent large-cohort investigations. A significant Danish cohort study discovered that while drinking-water nitrate in particular was linked to significantly higher rates of colon cancer, total dietary nitrate and nitrite did not show any association with bowel cancer when examined collectively. This finding would have been undetectable in any analysis that treated "dietary nitrate" as a single undifferentiated exposure variable. Similarly, a 2025 ovarian cancer cohort study treated source-stratification as a methodological requirement rather than an optional refinement, explicitly modelling distinct hazard ratios for dietary nitrate, dietary nitrite, plant-source nitrite, animal-source nitrite, and processed-meat-source nitrite (Ammons et al., 2025).

4. ENDOGENOUS FORMATION PATHWAY, GASTROINTESTINAL MECHANISM

4.1. Gastric nitrosation environment

The first process involves a direct chemical reaction between nitrite and secondary amino compounds. This reaction is very sensitive on pH and does not occur quickly at neutral pH, even when chemical catalysts are present. In the typical, acidic (pH ~1–3) stomach, nitrite protonates to nitrous acid, producing the nitrosonium and NO^3 species that subsequently react with dietary secondary amines. This is the classical mechanism explained in Section 2. Normal stomach acidity is necessary for this pathway to work well (Leach et al., 1987). Strangely, when gastric acidity is decreased, a second, mechanistically separate pathway takes over. This second process progresses far more quickly at neutral pH than the chemical reaction and relies on direct bacterial catalysis of N-nitrosation, mediated by bacterial enzyme systems. Instead of being shielded by less acid-driven chemistry, the

achlorhydric or hypochlorhydric stomach opens a completely other creation route because bacteria may catalyse N-nitroso compound production at neutral pH in a way that the purely chemical pathway cannot (Vermeer et al., 2002). An established model of stomach carcinogenesis revolves around this bacterial route. According to Correa's theory, atrophic gastritis and hypochlorhydria lead to gastric bacterial colonisation, the conversion of dietary nitrate to nitrite, and the creation of potentially carcinogenic NOCs. Given the widespread use of acid-suppressive medications, this has direct clinical relevance. A controlled study of long-term PPI use found that intragastric pH was significantly higher in omeprazole-treated patients than in untreated controls. Urinary N-nitroso dimethylamine excretion was significantly higher in the PPI-treated group, but total intragastric volatile N-nitrosamine levels did not differ significantly between groups. This suggests that acid suppression may alter nitrosamine formation or absorption dynamics without necessarily increasing total intragastric NOC concentration. The evidence, however, is not entirely consistent. A different controlled trial discovered that a two-week induced increase in gastric pH in healthy, *H. pylori*-negative subjects increased intragastric nitrate-reducing bacteria but did not significantly increase nitrite or N-nitroso compound concentrations. These authors concluded that reduced gastric acid secretion is not a necessary or sufficient precursor to carcinogenic N-nitroso compound formation and that additional mechanisms must be invoked to fully explain gastric carcinogenesis (Vermeer et al., 2002). *H. pylori* have been a prime candidate to directly cause bacterial nitrosation due to its well-established role as a stomach carcinogen; nevertheless, the direct evidence is not as strong as is sometimes thought. A more recent, genetically diverse strain-panel study found that H. pylori does not induce formation of the carcinogenic compound N-nitroso morpholine in vitro and that its previously reported weak nitrosation capacity is insufficient to nitrosate even the relatively easy-to-nitrosate test amine used (Ziebarth et al., 1997). This suggests that the increased risk of gastric cancer associated with H. pylori colonisation is unlikely to result from *H. pylori* directly catalysing nitrosamine formation. Since a lot of secondary literature still suggests that *H. pylori* are a direct nitrosating agent, this is a really important detail for a review to specifically point out. The more accurate current position is that H. pylori probably increase the risk of gastric cancer thru inflammation and altered gastric physiology rather than direct catalytic nitrosation itself (Vermeer et al., 2002).

4.2. Colonic/microbial nitrosation environment

Although the stomach was identified as one endogenous nitrosation location in Section 4.1, a significant amount of N-nitroso compound synthesis takes place in the colon further downstream, driven by a process unique enough to permit its own research literature. Because bacteria in the large gut decarboxylate amino acids to make amines and amides which can subsequently be N-nitrosated in the presence of a nitrosating agent, NOCs are found in the colon and are formed endogenously. Because the local microbiota's protein fermentation constantly replenishes the substrate pool that nitrosation acts upon, colonic nitrosamine production does not require preformed dietary amines at all (Bingham et al., 2002). The heme-iron dose-response effect is the most robust and reliably reproduced discovery in this literature. Eight male volunteers in controlled-feeding studies showed a significant three-fold increase in faecal NOCs, from about 40±7 to 113±25 micrograms per day. This exposure range is comparable to faecal exposure from tobacco-specific NOCs in cigarette smoke. Additionally, there was a significant increase in faecal excretion of the promoter ammonia. Subsequent research verified that this dose-relationship is consistent: in a series of controlled tests involving volunteers, researchers examining the hypothesis that

higher nitrogenous residues from red meat would raise endogenous N-nitrosation discovered a consistent dose response to red meat consumption. Since then, the catalytic process has been elucidated at the molecular level: heme increases the production of nitroso compounds in the gastrointestinal tract, with S-nitroso thiols and nitrosyl heme being the primary nitroso compounds generated in the GI tract (Foods & 2019).

4.3. Secondary amines and amides availability from dietary protein

Secondary amines are widely but unevenly distributed in foods, according to systematic surveys. The most common secondary amines found in food surveys, aside from dimethylamine and diethylamine, were pyrrolidine, piperidine, N-methylbenzylamine, N-methylaniline, and N-methylphenethylamine, with the latter being the most common secondary amine in foods derived from plants. The majority of foods have low secondary amine concentrations, but there are noticeable outliers. Herring preparations, some cheeses, and radish samples had significantly higher concentrations, with red radish having the highest recorded content at 38 ppm pyrrolidine, 20 ppm pyrroline, 5.4 ppm N-methylphenethylamine, and 1.1 ppm dimethylamine (Neurath et al., 1977). A significant amount of nitrosatable substrate is produced metabolically after consumption; not all of it is preformed in food. While food industry research has described how proteins and free amino acids are metabolised into biogenic amines like agmatine, spermine, spermidine, putrescine, and cadaverine via decarboxylation, none of the common amino acids or these resulting biogenic amines actually carry a DMA functional group. This means that some additional metabolic step, rather than direct decarboxylation alone, is required to generate DMA-type nitrosamine precursors from the majority of dietary amino acids (Bei et al., 2023). Since proline's chemistry directly connects the kinetic results from Section 2 to actual food intake, it merits special attention. Proline is structurally unique among the twenty naturally occurring proteinogenic amino acids because, when incorporated into proteins, its secondary amine forms a tertiary amide. However, as a free amino acid, its secondary amine is still accessible for nitrosation, which is consistent with previous kinetic modelling demonstrating that pyrrolidine-ring amines have an exceptionally low nitrosation activation barrier (Section 2.3). Nitrosation-chemistry literature directly supports this: amino acids with secondary amine functionalities, such as proline and sarcosine, form stable nitrosamine products, and nitrosation efficiency generally decreases with increasing distance between an amino acid amine group and its carboxylate group (Mothes et al., 2013).

4.4. Microbiota contribution to nitrate reduction and nitrosation promoting enzyme

The enzyme systems that function at every stage of the nitrate-nitrite-nitrosation process, the oral and gut microbiota actively regulate nitrosation rather than merely facilitating it. The major gut-level microbial effect appears to be detoxifying rather than promotive, which should temper any framing of "the microbiota" as a uniform risk factor. This section summarises that regulatory role and highlights a point of true subtlety for your evaluation.

Oral nitrate-reducing bacteria use nitrate in saliva as an alternative electron acceptor to oxygen, reducing it to nitrite via the enzyme nitrate reductase (Hager et al., 2026). This reduction is not incidental, but bacterially necessary; dietary nitrate consumption starts "nitrate bathing" of nitrate-reducing oral microbes, causing oral nitrate reductase activity as the first enzymatic step in the entire enterosalivary pathway outlined in Section 3.2. This phase is functionally load-bearing: the oral step is rate-limiting for downstream nitrogen oxide production, not just a passive relay, as demonstrated by the measurable reduction of stomach NO synthesis caused by a 7-day antiseptic mouthwash that lowers oral nitrate reductase activity (Chai et al., 2024). Outside of the

mouth, nitrate reductase enzymes are part of three different bacterial systems: cytoplasmic assimilatory reductases (Nas), cytoplasmic nitrate reductase (Nar), and periplasmic nitrate reductase (Nap). These systems feed into three main bacterial nitrate reduction pathways: respiratory denitrification, assimilatory nitrate reduction, and dissimilatory nitrate reduction to ammonium (DNRA). Nitrite reductase (Nir), NO reductase (Nor), and nitrous oxide reductase (Nos). Which pathway predominates and what end product is produced depends on the species composition. For example, denitrifying species like Neisseria and Rothia are associated with higher systemic NO levels, while DNRA-favoring organisms like Prevotella and Veillonella are associated with lower systemic NO levels. This means that the composition of the oral/gut microbial community, rather than just the total bacterial load, determines how much nitrite versus other end-products a given dietary nitrate intake (Chai et al., 2024). The most important new discovery for this review is that the primary enzymatic involvement of the intestinal microbiota in nitrogen metabolism seems to be protective rather than nitrosation-promoting. The human gut microbiota prevents the formation of carcinogenic nitrosamines by reducing nitrate and nitrite to ammonia or NO (Berends et al., 2019). A 2026 study that directly characterized this activity across dominant gut phyla found that Escherichia coli demonstrated the highest nitrite/nitrate-reducing efficiency of the bacteria tested, indicating a key role in detoxification and prevention of nitrosamine formation. Bacteroides and Phocaeicola species also contributed significantly to nitrite reduction, with collective stool sample activity totalling about 620 μmol $NO2^{-}$ reduced per hour, indicating that colonic nitrite concentrations should normally remain very low. The risk of microbial nitrosation is moved and conditioned by this protective frame, but it is not eliminated. This subsection is directly related to the achlorhydria/bacterial-nitrosation route covered in Section 4.1 because in cases of oral dysbiosis, nitrate rather than nitrite is more likely to reach the lower bowel intact. This means that the majority of colonic nitrite in that scenario comes from bacterial enzyme activity within the intestine itself, altering local nitrogen metabolism. According to that pathway, nitrosation-promoting versus nitrosation-suppressing microbial activity is a context-dependent result shaped by local pH and the presence of inhibitory dietary compounds. This is further supported by the fact that the amount of nitrite produced from nitrate metabolism depends on reaction mechanisms, compound concentrations, and the pH of the local environment (Yang et al., 2025).

5. MODULATOR: INITIATOR AND PROMOTOR

Nitrosation inhibitors and promoters operate through the same chemical bottleneck involving the reactive and electrophilic nitrosating intermediates (NO^{+}/N_2O_3) discussed in Section 2. Inhibitors include vitamin C, α-tocopherol, polyphenols, and competing thiols. They inhibit the reaction either by reacting with the nitrosating intermediate itself or directing them to non-carcinogenic products (S-nitrosation) prior to nitrosation of the secondary amine. On the other hand, promoters including heme iron, thiocyanate, carbonyls, and nitrosation-promoting bacteria in the gut catalyze the reaction or increase the amount of nitrosatable substrate. Consequently, they increase the formation of N-nitrosamines using the same amount of nitrite. Since these opposite effects occur on the same rate-limiting step, the amount of nitrosation product will depend more on the ratio of nitrosation inhibitors to promoters present rather than the amount of nitrite. Table 2 shows the major compounds in each class along with their mode of action and examples in the diet.

Table 1. Nitrosation Inhibitors and Promoters: Mechanism, Potency, and Source

Agent	Category	Specific mechanism	Quantitative effect	Dietary/matrix source	Reference
Ascorbic acid (Vitamin C)	Inhibitor	Reduces HNO_2/NO^+ to NO before amine reaction; water-soluble, acts in aqueous phase	~90% inhibition of NPYR and ~80% inhibition of NDMA formation in fried bacon	Citrus fruits, vegetables; added as sodium ascorbate (E301) in commercial curing	Shah & Mitch, 2012
α-Tocopherol (Vitamin E)	Inhibitor	Scavenges nitrosating agents in lipophilic phase; synergistic with ascorbate across aqueous+lipid phases	Significantly reduced NPYR in fried bacon; effect stronger when combined with sodium ascorbate than alone	Vegetable oils, nuts, seeds	Lathia et al., 1989
Flavanols (procyanidin B2, epicatechin)	Inhibitor	Direct reaction with HNO_2 via vicinal phenolic hydroxyl groups	Procyanidin B2 inhibited NDMA formation ~2-fold more effectively than epicatechin alone	Tea, cocoa, apples, grapes	Sirvins et al., 2024
Hydroxycinnamic acids (caffeic acid, ferulic acid)	Inhibitor	Requires simultaneous phenol group + olefinic bond for potency (structure-activity determined)	Potent inhibitors (caffeic, ferulic, catechin) vs. weak ones (cinnamic, vanillic acid) activity structure-dependent, not universal to all phenolics	Coffee, whole grains, fruit	Y. Zhao et al., 2021
Thiols (cysteine, glutathione)	Inhibitor (pathway diversion)	Competes for nitrosating species via S-nitrosation, diverting flux away from N-nitrosation	Mechanistic diversion rather than destruction reduces N-NOC yield proportional to thiol: amine ratio	Allium vegetables, egg, protein-rich foods	Broniowska & Hogg, 2012
Heme iron	Promoter	Catalyzes GI N-nitroso compound formation; forms nitrosyl heme and S-nitrosothiols as main products	3-fold increase in fecal NOC (40→113 µg/day) when red meat intake increased from 60→600 g/day	Red and processed meat	Bingham et al., 2002
Thiocyanate	Promoter	Catalyzes nitrosation specifically under acidic conditions; secreted into gastric juice via salivary glands	Salivary thiocyanate: 5.5±0.2 mM in smokers vs. 1.8±0.2 mM in non-smokers smoking status is a direct confounder	Cruciferous vegetables (minor dietary source); endogenous, smoking-elevated	Hinuma et al., 1990
Carbonyl compounds (formaldehyde, related aldehydes)	Promoter	Forms carbinolamine/iminium intermediates that react preferentially with N_2O_3	5- to 152-fold increase in N-nitrosamine formation depending on specific secondary amine, at physiological Ph	Maillard-reaction products in cooked/fried/browned foods	
Nitrosation-permissive gastric microbiota	Promoter (conditional)	Bacterial catalysis of N-nitrosation dominates at neutral pH, unlike the chemical pathway which requires acidic pH	Rate of bacterial catalysis exceeds chemical nitrosation specifically under hypochlorhydric/achlorhydric conditions	Endogenous gastric flora; conditional on host acid status, not a food source	Oh et al., 2025

6. QUANTIFYING EXPOSURE: ANALYTICAL AND MODELING APPROACHES

6.1. Fecal and urinary NOC Biomarkers

The inability to measure endogenous nitrosation directly led to the use of indirect measurements of nitrosative exposures using excretion-based biomarkers of exposure including fecal apparent total N-nitroso compounds (ATNC) as indirect measures of nitrosation in the colon, and urinary NOC as an indirect measure of nitrosative exposure via absorption. The sensitivity of the test was shown in animal tests whereby rats were administered with various doses of sodium nitrite in their drinking water (Li et al., 2022). Urinary apparent NOCs excretion varied from 0.9 nmol/day in non-exposure conditions to 37 nmol/day in the maximum exposure condition, which was 1,000 mg NaNO2 per liter of drinking water, with excretion achieving its maximum value during the first day of exposure and subsequently declining. As can be seen from the results above, this biomarker is highly sensitive to nitrite exposure in terms of rapid reversibility. Faecal ATNC is especially valuable as a biomarker since it is associated with a downstream genotoxic endpoint (Xu et al., 2015). The formation of O6-carboxymethylguanine (O6-CM-Gua) DNA adducts in colonic exfoliated cells from healthy volunteers is positively correlated with the red meat intake, and O6-CM-Gua concentrations are positively correlated with apparent total fecal N-nitroso compounds (ATNC) concentrations. The exposure assessment technique discussed above is directly related to the DNA alkylation process. The panels of urinary biomarkers provide greater site specificity compared to the fecal ATNC concentration. In a nested case control study in the Shanghai Cohort, the concentrations of urinary nitrate, nitrite, N-nitroso-2-methylthiazolidine-4-carboxylic acid (NMTCA), N-nitroso proline (NPRO), N-nitroso sarcosine (NSAR), and N-nitrosothiazolidine-4-carboxylic acid (NTCA) along with serum *H. Pylori* antibody status have been measured among 191 gastric cancer patients and 569 controls. In subjects who were negative for *H. pylori* antibodies, increased urinary nitrate concentrations were positively associated with gastric (Hebels et al., 2011). Ingested NDMA estimated using a validated food frequency questionnaire as opposed to a direct measure using a urine test in a large-scale cohort study has led to some of the more reliable cancer risk factors observed in the literature. Specifically, in the EPIC-Norfolk study, dietary NOC was associated with increased gastrointestinal cancer risk, especially rectal cancer risk, while showing that there was an interaction between the plasma vitamin C concentration and NDMA intake with regard to cancer risk, thus validating the inhibitor model described in Section 5 in the real world rather than *in-vitro*. A separate case-control study in Canada found that NDMA intake is related to increased colorectal cancer risk, specifically rectal and proximal colon tumors and not colorectal (Zhu et al., 2014).

6.2. PBPK modelling methodology

The physiologically based pharmacokinetic (PBPK) modelling resolves the main issue previosly excretion-based biomarkers cannot distinguish between endogenously produced NOCs and preformed NOCs ingested with diet. The PBPK model uses compartmental structure for describing the absorption, distribution, metabolism, and excretion (ADME) of a particular chemical and is common practice in toxicology as a tool for the risk assessment of internal exposure and chemical transformation (Shi et al., 2026). However, when applying PBPK modeling specifically for nitrite and nitrate, this approach becomes obligatory due to the fact that saliva adds nitrite through the conversion of about 25% of oral nitrate through oral flora, which means that nitrosamine formation should be calculated based on the intake of both nitrite and nitrate, not separately, and the PBPK model can model this transformation in one process. Previous research in this field used both compartmental modeling and *in-vitro* simulation; Lin et al. (2020) performed

PBPK modeling to estimate the endogenous exposure of nitrate/nitrite from vegetables, and Zeilmaker et al. (2010) performed the simulation of stomach-level NDMA formation using a dynamic *in-vitro* gastrointestinal model (Kang et al., 2024; Shi et al., n.d.).

6.3. Limitation of concentration-based vs mechanism-based exposure estimation

It is already established that endogenous NDMA exposure could be greater by two to three orders of magnitude than preformed NDMA exposure. The implication of this for regulation is much more serious than it seems at first blush, since concentration-based assessments of exposure are not only inaccurate but structurally unable to measure the exposure route, as it was never meant to do so (Kotopoulou et al., 2022). The 2023 opinion of the EFSA CONTAM Panel on risk associated with preformed dietary N-nitrosamines evaluated via margin-of-exposure methodology was entirely focused on preformed N-nitrosamines already formed in foods at the time of ingestion. Inherently, this methodology can never pick up the route of exposure shown in Sections 2 and 4 to be the major route which means that a food product might meet every regulatory standard set based on concentration thresholds while being the primary source of nitrosamine exposure for the consumer via post-consumption chemistry. The second limitation is related to the source attribution rather than to the total concentration. The large French cohort study made a distinction between the two different sources of nitrite/nitrate exposure – food additives and natural dietary and water sources rather than taking “the total nitrite intake” as a single and undifferentiated exposure variable (Cheng et al., 2024). The study used repeated 24-hour dietary records coupled with a food composition database allowing the identification of nitrite coming from food additives through the use of commercial names of products. The NutriNet-Santé cohort (104,168 adults, median follow-up 6.7 years) observed the positive association of total nitrites (HR tertile 3 vs. 1 = 1.29, 95% CI 1.06-1.56) and natural nitrites (HR tertile 3 vs. 1 = 1.27, 95% CI 1.05-1.54) with increased risk of type 2 diabetes. Meanwhile, those participants whose exposure to nitrites from food additives was increased demonstrated the specific risk pattern independent from the natural-source exposure (Srour et al., 2023). The above-mentioned source-resolved design is very important indeed since it is not typical for the majority of national exposure surveys which usually assess the aggregate exposure to the nitrite/nitrate against the Acceptable Daily Intake level. The most direct methodological solution to each of these limitations would come from the use of probabilistic toxicokinetic models, which combine the concepts of exogenous and endogenous exposure into one distributional estimate rather than considering them as two separately regulated concepts(Eisenbrand et al., 2024). Probabilistic risk and benefit assessment using the toxicokinetic model with an estimation of dietary exogenous exposure to nitrate/nitrite in conjunction with endogenously produced nitrite was performed based on a total diet study precisely because, as authors point out, risk and benefit assessment of nitrate and nitrite is complicated by the high inter-individual variation in exposure and endogenous production of nitrite that cannot be represented by concentration-based point estimates. The use of distributional method, which includes estimation of the entire exposure distribution rather than its mean/median value, is what Section 11 suggests should be adopted as the method of regulation in scientific terms, as there is no way a single ADI value can protect a high-endogenous-converter individual and represent population exposure level at the same time (Cheng et al., 2024).

7. TOXICOLOGICAL MECHANISM OF CARCINOGENICITY

Dietary nitrite's carcinogenic potential is not realized through the compound itself, but through its downstream conversion into NOCs capable of chemically damaging

DNA. This section addresses that mechanistic link directly how these compounds are metabolically activated into DNA-reactive electrophiles, which specific DNA adducts result and why they are mutagenic, and why the resulting damage shows marked tissue specificity rather than affecting the body uniformly.

7.1.DNA alkylation and O6-methylguanine adduct formation

However, n-nitrosamines cannot cause the induction of cancer on their own. They have to be subjected to metabolism in order to become able to inflict harm on DNA. All n-nitrosamines have to be metabolized before becoming carcinogenic. It is because electrophilic metabolites of n-nitrosamines can easily alkylate DNA and thus start causing carcinogenesis. As far as the NDMA activation goes, this is achieved via well-studied α-hydroxylation process. This activation is done in the target organs like the liver where NDMA is transformed into the hypothetical methyldiazonium ion which causes alkylation of DNA bases and phosphoribose backbone (Li et al., 2022). The activation pathway includes abstraction of the α-hydrogen atom by NDMA followed by reaction with hydroxyl radical to form α-hydroxy-NDMA, which decomposes into formaldehyde and methyl diazonium ion – the electrophilic molecule responsible for alkylation of DNA. As can be seen, the activation of NDMA through CYP2E1 is responsible for the tissue specific NDMA carcinogenicity mentioned in Section 7. Centrilobular hepatocytes appear to be more susceptible not only because of being the initial target for the alkylating agent but also because of the high level of CYP2E1 expression in comparison to other organs (e.g. lungs and kidneys), where this enzyme is expressed at a low level. Adducts formed as a result have different structure, and adduct specificity defines the outcome (Armijo et al., n.d.). Addition of NDMA to DNA results in the formation of 7-methylguanine (m7G, ~65–70% of total adducts formed), a methyl phosphotriester backbone adducts (~15%), O6-methylguanine (m6G, ~7%), and 3-methyladenine (m3A, ~3%), among others quantitatively insignificant base adducts. Even though accounting for a small part of the total number of adducts formed, O6-methylguanine and 3-methyladenine are regarded as the most biologically important of all those formed by NDMA, owing to their known ability to (Chen et al., 2021). Here lies the key mechanistic message for any carcinogenesis-focused review to impart: The risk of cancer resulting from exposure to N-nitrosamines is largely due to the activity of a few adducts, rather than the overall level of DNA damage resulting from the exposure – metrics of adduct levels will underestimate the risk posed by the dose-specific mutagenicity of N-nitrosamines. O6-methylguanine is mutagenic because it mispairs during replication (Snodin et al., 2024). In the absence of repair, the O6-methylguanine lesions will pair with thymine rather than cytosine during replication, resulting in a G:C->A:T transition mutation which, if not corrected, gets permanently incorporated into the genome and constitutes an identifiable mutational signature for exposure to alkylating agents in mutational spectra analyses. Here we are going to introduce a cellular defense mechanism that is pertinent to individual susceptibility to such alkylation damage the O6-methylguanine-DNA methyltransferase (MGMT), a DNA repair enzyme that can remove alkyl adducts at the O6 site of guanine, thus eliminating the particular damage generated by NDMA metabolism. The fact that MGMT activity varies greatly among different individuals and tissues and that its levels are depleted under conditions of heavy or prolonged alkylation makes MGMT a potential though poorly understood predictor of susceptibility to the damage in question (Chen et al., 2021). The supporting toxicological data is particularly strong in support of biological relevance for this pathway. Both NDMA and similar N-nitrosamines have been found to induce tumors in a large number of animal species; NDMA was shown to be tumorogenic in 16

animal species while N-nitrosodiethylamine caused tumor in 26 species, including rainbow trout and cynomolgus monkeys (a cross-species database for chemical carcinogenesis). This cross-species reproducibility together with the previously described CYP2E1-dependent metabolic activation of NDMA and subsequent O6-methyguanine mispairing represents one of the key reasons why the toxicological plausibility of dietary nitrites-induced NDMA carcinogenicity in humans is considered to be mechanistically well-established, despite the absence of epidemiological evidence described in Section 8 (Xu et al., 2024).

8. EPIDEMIOLOGICAL EVIDENCE

8.1. Colorectal cancer

Colorectal cancer has been the best studied cancer site with regard to the effects of nitrate and nitrite intake, yet the aggregate evidence is still highly heterogeneous, and we believe this issue is due to source-blind modelling of exposures but not the absence of biological effects. According to the results of a systematic review and meta-analysis of fifteen prospective cohort and case-control studies involving 2,573,524 subjects and 38,848 cases of colorectal cancer, dietary nitrate intake was linked to increased risk of colorectal cancer (pooled hazard ratio [HR]: 1.13; 95% confidence interval [CI]: 1.04-1.23; $I^2 = 38\%$), while dietary nitrite was not associated with the disease risk (pooled HR: 1.07; 95% CI: 0.95-1.21; $I^2 = 61.6\%$), and drinking-water nitrate was also not significantly associated with the disease risk (HR: 1.04; 95% CI: 0.92-1.19; $I^2 = 64.7\%$) (Depeursinge et al., 2010). The high heterogeneity in both null associations, in excess of 60%, is important on its own since it means that the pooling of studies which do not stratify the sources of exposures does not clarify the effect but, instead, hides it. The Danish Diet, Cancer and Health cohort in 2025 is an example of when such approach results in missing the true effect. The cohort included 54,610 subjects with follow-up during 24.7 years and 2,245 cases of colorectal cancer who had no association between nitrate and nitrite intake from additive-permitted meat sources and the incidence of the disease. However, the authors' explanation of this negative association is crucial for understanding it properly: the additive-permitted meat sources were responsible for only 0.5% and 3.9% of dietary nitrate and nitrite intake, respectively, so this negative association is due to insufficient variability in the exposure (D. Erichsen et al., n.d.). In addition, this study identified another confounder which is directly related to source-based risk modeling: nitrate and nitrite in the foods of animal origin (e.g., dairy products) did not contain the accompanying amines and, therefore, were less likely to form NOCs and even had the inverse association with colorectal cancer risk (Shi et al., 2026).

8.2. Gastric cancer

Gastric cancer has shown stronger evidence of a specific nitrite signal than colorectal cancer, with one of the clearest source-based discrepancies in the whole body of dietary nitrate/nitrite research a striking difference in the direction of association between dietary nitrate and nitrite within the same disease. A meta-analysis of 22 publications including 49 studies on nitrate/nitrite (19 on nitrate, 19 on nitrite, 11 on NDMA) reported a summary relative risk for gastric cancer of 0.80 (95% CI: 0.69–0.93) for dietary nitrate, 1.31 (95% CI: 1.13–1.52) for dietary nitrite, and 1.34 (95% CI: 1.02–1.76) for NDMA specifically (Erichsen et al., 2026). Such a pattern a modest inverse association for dietary nitrate, but positive associations for dietary nitrite and NDMA is one of the best quantitative proofs from the whole body of research that nitrate and nitrite cannot be grouped into one exposure variable, since their association with the same cancer endpoint is opposite in sign. In fact, in a later dose-response meta-analysis up to April 2024, a significant linear positive association between the risk of gastric cancer and high nitrite consumption (linearity $P = 0.001$) was shown, while no significant association between gastric cancer risk and dietary nitrate intake was

found in either case-control or cohort studies (Catalano et al., 2009).

The site-specific analysis of gastric cancer is even more detailed. A systematic review and meta-analysis showed a positive association between NDMA intake and gastric non-cardia adenocarcinoma (HR per 0.1 µg/day increase: 1.06, 95% CI: 1.01–1.10, p-trend = 0.09) risk in men, along with an equally sized association with oesophageal squamous cell carcinoma (HR: 1.15, 95% CI: 1.05–1.25, p-trend = 0.01) (Long et al., 2025) . At the same time, it is worth noting that most of the individual studies reviewed did not find any statistically significant associations, and there was no association between nitrate/nitrite and oesophageal or gastric cancer in a ten-year U.S. cohort thus, showing that pooled meta-analytic results do not eliminate substantial heterogeneity between individual studies, which may very well be caused by the lack of source stratification. In 2026 Danish cohort study addresses this heterogeneity directly, stratifying the risk of gastric cancer by source (plant, naturally-occurring animal, and additive-permitted meat sources) and the proxy of H. pylori infection status in a cohort of 54,610 patients. Such stratification design directly tests two mechanistic hypotheses previously.

8.3. Esophageal and pancreatic cancer

The weakest and least consistent evidence in this review comes from the relationship between oesophageal and pancreatic cancer and nitrite consumption. This is a clear contrast to the more consistently established risk factor for gastric cancer. The results of a dose-response meta-analysis of literature up to April 2024 found no correlation between nitrite intake and the risk of developing oesophageal cancer in case-control studies (OR: 1.48, 95% CI: 0.91-2.42, P= 0.12). There were eighteen studies conducted in 1994 - 2017 years (Nitric Oxide, 2024). In 2025, a meta-analysis of studies specifically of plant-based nitrate and nitrite also found no effect in terms of oesophageal cancer (Xie et al., 2016). Pooled oesophageal cancer estimates for high nitrate intake (RR: 0.65, 95% CI: 0.39-1.08) and high nitrite intake (RR: 0.96, 95% CI: 0.48-1.91) are statistically insignificant with wide confidence intervals because of the relatively small number of studies. The important exception in this null literature is the study of NDMA, rather than total nitrite consumption. Dietary NDMA intake was found to be a risk factor of oesophageal squamous cell carcinoma (HR per 0.1 µg/day increase: 1.15, 95% CI: 1.05-1.25, p-trend = 0.01), which again reinforces the already noted finding about NDMA as a specific compound and more consistent associations than total nitrite or nitrate. Pancreatic cancer appears to be the most consistently null type of cancer in relation to nitrate and nitrite consumption (Song et al., 2015). In an earlier meta-analysis of observational studies, no association between dietary nitrate or nitrite intake and pancreatic cancer risk has been found. Other types of cancer, such as breast, bladder, renal cell system, and non-Hodgkin lymphoma, also showed null findings. The current evidence has not changed this finding. The 2025 meta-analysis of digestive cancers has considered only two studies related to pancreatic cancer; the evidence base is too small for the pooled estimation. Also, there is no association of ingested nitrate, nitrite, and disinfection by-products with pancreatic cancer risk in a large postmenopausal cohort. It is better to understand this pattern across both cancer sites as an evidentiary gap than a lack of any danger. Unlike colorectal and gastric cancers, where the analysis of NDMA and nitrite separately from total nitrate/nitrite intake revealed some associations otherwise hidden in pooled data, the evidentiary base of esophageal and pancreatic cancer (Ghasemi et al., 2024).

9. RESEARCH GAPS AND FUTURE DIRECTIONS

In light of the mechanistic evidence laid out above, the three gaps in current risk assessment that have emerged can be succinctly described as follows; First, there is the problem of individual variation that is

currently considered 'noise' rather than 'signal' by contemporary models. In particular, gastric pH status creates a dichotomy between two chemically distinct nitrosation modes: direct acidic nitrosation in the acidic stomach, and bacterial-catalysed nitrosation that becomes predominant under the condition of hypochlorhydria/achlorhydria, including proton pump inhibitors use (Long et al., 2025). This is compounded by the independent role of microbiome composition, as oral and gut bacteria differ in terms of preference of denitrifying versus nitrifying pathways, thus influencing systemic NO levels, and the efficiency of colonic detoxification is known to vary depending on the bacterial phylum in question (Hager et al., 2026). Third, and this is the effect that occurs downstream of exposure, MGMT repair capacity provides selective protection against NDMA genotoxicity, but not structurally different nitrosamines, such as N-nitrosodiethanolamine and N-nitrosomethylaniline. Therefore, two individuals with equal exposure modeled by current risk assessment methods are subject to a very different risk of developing cancer because of which specific compound dominates in their diet, and what repair capacity they have (Long et al., 2025). None of the above three sources of individual variation is included in contemporary population risk models which apply the same exposure-risk function to all consumers. The above-described gap has an important practical implication for food labelling regulations. Current regulations provide information on concentration of nitrite/nitrate in a foodstuff, and no information on its nitrosation potential is available. This means that the consumer has no idea about the presence of additional inhibitors of nitrosation or promoters, the heme iron content, or even what the nitrosamine yield will change due to the frying temperatures or storage time, the latter having been shown to influence NDMA content by 40% (Xie et al., 2016). Developing a nitrosation-potential index, where the ratio of the inhibitors to the promoters is taken into account, would be more informative than the current system and would provide an incentive for the producers to increase that ratio, rather than decreasing the nitrite content only. Development of such index, preferably through standardized *in-vitro* assays simulating gastric conditions of nitrosation, is a realistic goal. The key gap, however, is regulatory rather than analytical. Current systems, such as the European Union's 2023 nitrite regulations and the Margin of Exposure methodology of the European Food Safety Authority, evaluate preformed dietary nitrosamine content alone, even though there is evidence that *in-vivo* NDMA content could be two or three orders of magnitude greater than preformed dietary content. No regulator has yet required or officially implemented physiological modelling as part of their risk evaluation process, despite having already shown its power to detect cancer links via national cohort analysis where concentration-based measurement methods had proven incapable (Shi et al., 2026). Establishing consistency across regulatory authorities in how they implement this methodology, deciding which nitrosamine species to include in the models, applying the individual variability data described above, and translating this information into concrete concentration or labelling regulations represents the most impactful unmet need that this review can identify. The ultimate consequence of the mechanistic model developed throughout this review will depend on whether regulatory science addresses this gap or continues to improve concentration measurement in a framework that the evidence suggests is measuring the wrong quantity (Eisenbrand et al., 2024).

10. CONCLUSION

Nitrite intake must be assessed not merely as a preservative additive or the level of preformed nitrosamines in food, but rather as an endogenous precursor in an elaborate network of nitrate-nitrite-nitrosation balance.

The information presented above suggests that endogenous N-nitrosamine synthesis is controlled by gastric pH, availability of secondary amines and amides, heme iron, carbonyl groups, thiocyanate, dietary inhibitors, microbiotic flora, and the individual's ability to metabolize and repair DNA damage. Acid-induced nitrosation in the stomach and bacteria-induced nitrosation under hypochlorhydria condition are two different mechanisms, while oral and intestinal microbiota might contribute to the reduction of nitrate and production of nitric oxide, as well as perform nitrosation in response to environmental factors depending on species and conditions. Carcinogenicity of NDMA is feasible from a biological standpoint via metabolism through the activation of CYP enzymes and producing DNA-alkylating intermediates such as O6-methylguanine, however, epidemiological associations are inconsistent and strongly depend on exposure route, matrix, confounders, and study design. Existing methodologies for assessing risk that are based mainly on preformed compounds may overlook the risk posed by post-ingestive formation and inter-individual variability. In future studies, source-resolved dietary intake assessments, NOC-related biomarkers in urine and feces, DNA adducts, dynamic gastrointestinal models, and probability-based PBPK models taking into account endogenous conversion should be considered. The development of standardized methods of nitrosation capacity and regulations that would take into account the balance between inhibitors and promoters would provide better consumer information and food safety measures. It is crucial to keep in mind that any mitigation measures need to maintain the beneficial effect of nitrites in preventing botulism while lowering unnecessary nitrosation promoters. In general, an integrated methodology taking into account mechanisms, sources, and physiology is needed for better understanding cancer risks.

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